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Morphology, ontogeny, and molecular phylogeny of two novel bakuellid-like hypotrichs (Ciliophora: Hypotrichia), with establishment of two new genera

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Abstract

The morphology, ontogeny and molecular phylogeny of *Apobakuella fusca* gen. n., sp. n. and *Parabistichella variabilis* gen. n., sp. n., from south China were investigated. *Apobakuella fusca*, brown colored, demonstrates bakuellid-like infraciliature, and a similar ontogenesis as the genus *Bakuella*. It is argued, however, that this species represents a novel genus, *Apobakuella*, which is characterized by two or more marginal rows on the right, several buccal and parabuccal cirri, and lack of frontoterminal and caudal cirri. Phylogenetic analysis based on SSU rRNA gene sequences supports the close relationship of *Apobakuella* with *Neobakuella* and *Diaxonella* within the core Urostylida. By contrast, *Parabistichella variabilis* has a dominant frontoventral row, few midventral pairs, a long midventral row, and one marginal row on each side. Its morphogenesis exhibits: (1) partial reorganization of the parental adoral membranelles; (2) over six frontoventral-transverse cirri anlagen; (3) intrakinetal development of the midventral row; and (4) very likely, formation of the frontoventral row from the midventral row anlage. Both the morphological characteristics and the SSU rRNA gene sequences suggest that it is incertae sedis among the basal hypotrichs. Further investigation of key taxa with additional molecular markers is required to reveal a better understanding on the phylogeny of *Parabistichella*.

Keywords: *Apobakuella*; Bakuellid; Hypotrichs; Morphogenesis; SSU rRNA; *Parabistichella*

Introduction

The ciliate subclass Hypotrichia Stein, 1859, has consistently been a focus of ciliatological research and now contains over 1000 nominal species which exhibit extremely diverse morphological/morphogenetical features (e.g. Berger 1999, 2001, 2006, 2008, 2011; Chen et al. 2010b; Foissner et al. 2004, 2010; Hu et al. 2010; Huang et al. 2010; Kamra and Kumar 2010; Li et al. 2010b; Schmidt et al. 2007; Vd'ačný et al. 2010).

The family Bakuellidae Jankowski, 1979 differs from other Urostylida members (e.g., Holostichidae or Urostylidae)

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in having three frontal cirri and a midventral complex composed of anterior midventral pairs and posterior midventral row(s) (Berger 2006; Jankowski 1979). The family contains at least eight genera, i.e., *Australothrix*, *Bakuella*, *Birojimia*, *Holostichides*, *Metaurostylopsis*, *Neobakuella*, *Parabirojimia* and *Paragastrostyla* (Berger 2006; Li et al. 2011). Several brief reviews of this family have been made in the past decades (Chen et al. 2010a; Eigner 1994; Eigner and Foissner 1992; Franco et al. 1996; Song et al. 2011), including a recent update in Lynn's (2008) classification scheme wherein Bakuellidae is a synonym of Urostylidae. Bakuellids are comparatively rare, and molecular data are available for only a few taxa (Li et al. 2011; Song et al. 2011; Yi et al. 2008a,b). In particular, the phylogenetic incongruence between the morphological and molecular data of some of its genera (e.g., *Metaurostylopsis* and *Parabirojimia*) challenges our understanding of the system and classification of the family Bakuellidae (Song et al. 2011; Yi et al. 2008a).

In this work, we describe two novel bakuellid-like hypotrichs with highly differentiated frontal cirri and midventral complex. We argue that they represent two new genera (*Apobakuella* gen. n. and *Parabistichella* gen. n., respectively) and that they are possibly able to be distinguished from each other at family level. Their morphology, ontogeny, and molecular phylogeny are investigated.

Material and Methods

Sampling and cultivation

Samples of *Apobakuella fusca* gen. n., sp. n. were collected from the surface of intertidal gravel in the Futian mangrove forest, Shenzhen, South China, on April 1, 2009, at a water temperature of 21 °C, pH 7.5 and salinity 14‰. Samples of *Parabistichella variabilis* gen. n., sp. n., meanwhile, were collected from the intertidal zone at the estuary of the Pearl River, Seagull Village, Guangzhou, South China, on April 14, 2009, at a water temperature of 20 °C and salinity 0.3‰ (freshwater). The surface sediments were transferred to Petri dishes with their original water and maintained as a raw culture in the laboratory for several days at room temperature. Rice grains were added to enrich the bacteria. About four days later, it was observed that the novel species, together with some euplotids and cyrtophorids, had become predominant.

Morphological and morphogenetic studies

Specimens were examined in vivo using bright field and Nomarski differential interference contrast microscopy. Protargol silver impregnation (Wilbert 1975) was applied to reveal the infraciliature and an ocular micrometer was used to measure the stained specimens. Drawings were made with the aid of a drawing device (Li et al. 2010a). To illustrate the changes during the morphogenetic process, parental cirri are depicted by contour whereas new ones are shaded black.

General terminology is mainly according to Lynn (2008). For an explanation of group-specific terms, see Berger (2006, 2008). The classification and nomenclature of hypotrichs follow Berger (1999, 2006, 2008).

DNA extraction, PCR amplification, and sequencing

Genomic DNA extraction, PCR amplification, and sequencing of the SSU rRNA gene were performed according to Jiang and Song (2010). The universal eukaryotic primers Euk A and Euk B (Medlin et al. 1988) were used to amplify the SSU rRNA gene. Cycling parameters were as follows: a pre-run of 5 min at 94 °C; then 35 cycles of 30 s at 94 °C, 1 min at 60 °C, and 2 min at 72 °C; and, finally, one cycle of 7 min at 72 °C. Sequencing in both directions was carried out on an ABI 3700 sequencer. Multiple clones (≥2) from the same PCR products were sequenced to verify the target gene. The SSU rRNA gene sequences were submitted to the NCBI/GenBank database, with accession numbers of JN008942 for *Apobakuella fusca* sp. n. (1766 bp) and JN008943 for *Parabistichella variabilis* sp. n. (1774 bp).

Phylogenetic analyses

In order to assess the phylogenetic positions of *Apobakuella fusca* gen. n., sp. n. and *Parabistichella variabilis* gen. n., sp. n., the SSU rRNA gene sequences were aligned using MUSCLE v3.7 (Edgar 2004), and trimmed using Bioedit v7.0.5.2 so that both ends were 1725 bp in length (Hall 1999). Preliminary Maximum Likelihood (ML) analyses were performed on CIPRES Portal V 2.0 (http://www.phylo.org) and showed similar topologies for different selections of taxa. We therefore selected a set of 52 species representing key taxa of hypotrichs for the phylogenetic analysis, with two choreotrichs and two oligotrichs as an outgroup. ML bootstrapping analyses were carried out using RAxML with the setting as described in Stamatakis (2006). Bayesian inference (BI) was performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) with the GTR+I+G model as selected by AIC in MrModeltest v.2.0 (Nylander 2004). Markov chain Monte Carlo (MCMC) simulations were run with two sets of four chains using the default settings: i.e. a chain length of 1,000,000 generations, with trees sampled every 100 generations. The first 25% of sampled trees were considered burn-in ones and were discarded prior to constructing a 50% majority rule consensus tree.